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(54) METHOD FOR REMOVING BITTERNESS OF PEPTIDE

(57)Abstract:
PURPOSE: To readily remove bitterness of peptides such as proline or aromatic amines by reacting a protein derived from an animal or a plant with a specific protease formulation.
CONSTITUTION: A protein derived from an animal or a plant is reacted with a protease formulation containing a prolyl endopeptidase or carboxypeptidase at 20-60°C (preferably 50° C) and pH4-7 (preferably pH5) for 4-5hr to carry out the objective removal of bitterness. Furthermore, the prolyl endopeptidase has the following physico-chemical properties. Relative activity of 0 for prolyl-p- nitroanilide when the carboxyl sides of proline in the peptide and protein are hydrolyzed and the hydrolytic properties for CBZ-Gly-Pro-pNa (CBZ is carbobenzoxy; pNA is p-nitroanilide) is 100, 0.29mM value of the substrate specificity (Km) for the CBZ-Gly-Pro-pNa, optimium pH; about 5, optimum temperature; 37°C, pH stability of ≥ 85% residual activity at pH4-7 when treated at 37°C for 2hr, etc.

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* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the reduction approach of the bitterness of the peptide by separating in a detail the proline which came after cutting by prolyl proteinase and came the carboxyl terminus of the proline which exists in a peptide to the carboxyl terminus of a peptide by the carboxyl peptidase, aromatic amino acid, and a branched chain amino acid about the clearance approach of the bitterness of a peptide.
[0002] The protease by which current various kinds are marketed in manufacture of a peptide is used. However, these proteases have the weak capacity to cut proline order. Therefore, although a proline will remain into a peptide, the peptide containing these prolines causes bitterness and poses a problem in manufacture of a peptide. Moreover, it is reported that the aromatic amino acid and the branched chain amino acid which exist in the carboxyl terminus of a peptide also caused the bitterness of a peptide.
[0003] About clearance of bitterness, reduction of bitterness is performed in production of the first peptide by changing the combination of the protease to be used by suppressing generating of bitterness or making various kinds of exoproteases act to the peptide which bitterness generated. However, there is a limitation in reduction of the bitterness by these approaches. Moreover, although the attempt which is going to separate the proline in the peptide by using carboxypeptidase, aromatic amino acid, and a branched chain amino acid is also made, by the approach of using only carboxypeptidase, there are many amounts of the separating amino acid and these amino acid that separated has an adverse effect on the taste of a peptide.
[0004]
[Problem(s) to be Solved by the Invention] This invention tends to offer the process of a peptide with little bitterness by using the protease pharmaceutical preparation containing prolyl proteinase and carboxypeptidase in view of there being a limitation on the occasion of reduction of the bitterness of a peptide by the conventional approach as mentioned above.
[0005]
[Means for Solving the Problem] According to this invention, the method of manufacturing the peptide with little bitterness by making the protease pharmaceutical preparation containing prolyl proteinase and carboxypeptidase act on the protein of the animals-and-plants origin is offered, and prolyl proteinase hydrolyzes the carboxyl side of the proline which exists in the following physicochemical property:(b) operation:peptides and proteins here.
(b) Substrate specificity : (1) The relative activity over the prolyl PARANITORO anilide at the time of setting hydrolysis activity over CBZ-Gly-Pro-pNA (CBZ : carbobenzoxy, -pNA:p-nitroanilide) to 100 is 0.
(2) Km values over CBZ-Gly-Pro-pNA are 0.29mM(s).
(c) Optimum Near [pH:5] (d) optimum temperature: 37-degree-C (e) pH stability: 37 degrees C shows 85% or more of residual activity in pH 4-7 the case where it processes for 2 hours.
(**) Temperature stability: In pH5, activity remains 80% or more by 52 degrees C and 1-hour

processing.
It ****.
[0006] this invention persons examined the approach of reducing the bitterness of the various peptides originating in the protein of animals and plants. Consequently, the structure in which the peptide with which the main causes of the bitterness of a peptide originate in a proline bent, The information depended on the branched chain amino acid which exists in the carboxyl terminus of a peptide, and aromatic amino acid that it is a thing is acquired. The proline which comes to the carboxyl terminus of a peptide by that cause except for the structure in which the peptide bent by cutting in enzyme the carboxyl side of the proline which exists in a peptide, Inference that reduction of bitterness was possible was reached by separating the branched chain amino acid and aromatic amino acid which exist in the carboxyl terminus of a peptide from the first by carboxypeptidase. However, since there was almost no capacity to cut the peptide linkage before and behind a proline and there was also no carboxypeptidase activity, the protease currently used conventionally finds out the approach of making GABI newly producing prolyl proteinase, carboxypeptidase, and a protease simultaneously, and producing a peptide with little bitterness by using the enzyme preparations obtained by this, and came to complete this invention.
[0007] The enzyme preparations used in this invention are an Aspergillus. It is the protease pharmaceutical preparation containing the prolyl proteinase and carboxypeptidase originating in ORIZE (Aspergillus oryzae) FS 1-32 (Fermentation Research Institute mycoparasite No. 12193).
[0008] Soybean protein, wheat protein, casein, etc. are used as protein of the animals-and-plants origin.
[0009] The reaction condition which hits carrying out this invention is as follows.
Reaction temperature: 20-60 degrees C (preferably 50 degrees C)
pH: 4-7 (preferably 5)
Reaction time: Although it changes with the substrate to be used, reaction temperature, pH, and amounts of enzymes, it is about 4 - 5 hours.
Enzyme concentration: It is a protease per 1g of substrates. 650PU extent carboxypeptidase About [0.01U] prolyl proteinase It is possible by increasing 0.03mU extent enzyme concentration more than this to shorten reaction time. Moreover, an enzyme addition can also be reduced instead of lengthening reaction time, and it is also possible to reduce the cost which starts an enzyme by this.
[0010] The enzyme activity used in the approach of this invention is searched for by the following approaches.
It asks by acting on the peptide which is the quality of an active group of prolyl proteinase, and carrying out the quantum of the hydrolysis reaction by the side of the carboxyl of a proline. It is measured by the enzyme activity indicated on these descriptions, and the following approach using CBZ-Gly-Pro-pNA as a substrate, and enzyme activity which separates the PARANITORO anilide of 1micro mol in 1 minute is used as one unit (U). CBZ-Gly-Pro-pNA decomposition activity-measurement method: They are 2mM(s) to 40% dioxane solution. Let what added 1ml (pH5.0) of 0.1M citric-acid-phosphoric-acid disodium buffer solutions to 0.25ml of things which dissolved CBZ-Gly-Pro-pNA be a substrate. 0.1ml of enzyme solutions after preheating is added, and this is made to react at 37 degrees C for 37-degree-C 10 minutes for 2 hours. A reaction is suspended with the 1M potassium chloride-hydrochloric-acid buffer solution (pH2) (stop solution) which contains 10% of Triton-X100 after a reaction, what made reverse sequence of adding the stop solution and an enzyme solution is used as the contrast solution, and an absorbance is measured in 410nm.
(CBZ : Carbobenzoxy)
[0011] It asks by carrying out the quantum of the hydrolysis reaction by the side of the carboxyl of the peptide which is the activity measurement substrate of carboxypeptidase. The enzyme activity indicated on these descriptions is CBZ-Glu-Tyr. It is measured by the following approach of using as a substrate, and enzyme activity which separates the thyrosin of 1micro mol in 1 minute is used as one unit (U). CBZ-Glu-Tyr decomposition activity-measurement method: Let 0.5 mM CBZ-Glu-Tyr / 1ml (pH7) of 0.05M phosphate buffer solutions be substrates. 50microl addition of the enzyme solution after preheating is done, and this is made to react at 37 degrees C for 37-degree-C 20 minutes for 60 minutes.

The absorbance in 570nm is measured by using as object liquid what added 500micro of ninhydrin reagents l after a reaction, cooled with the chilled water after heating for 15 minutes on the ebullition water bath, added 0.1M phosphoric-acid disodium / acetone 5ml, and added water instead of the enzyme solution. It applies to the standard curve which had created this beforehand, and the activity of carboxypeptidase is searched for from the amount of the thyrosin which separated.

[0012] It asks by carrying out the quantum of the amino acid which acts on the protein which is an activity measurement substrate of a protease, and separates. The enzyme activity indicated on these descriptions is measured by the following method of using casein as a substrate, and is using as one unit (PU) enzyme activity which separates the amino acid of the thyrosin equivalent of 1micro mol in 1 minute. Casein decomposition activity-measurement method: Let 1% casein / 5ml (pH7) of 0.05M phosphate buffer solutions be substrates. 1ml of enzyme solutions after preheating is added, and this is made to react for 10 minutes at 37 degrees C for 37-degree-C 10 minutes. 5ml of 0.44M trichloroacetic-acid solutions is added, and a reaction is filtered through the filter paper after neglect for stop and 37-degree-C 20 minutes. 5ml of 0.4M sodium-carbonate solutions and 1ml of Folin's reagents are added to 1ml of filtrate, and an absorbance is measured by 660nm after 37-degree-C 20-minute neglect. It applies to the standard curve which created this beforehand, and casein decomposition activity is searched for.

[0013]

[Example]

Added 0.166g of enzyme preparations containing prolyl proteinase 4.8 mU/g, carboxypeptidase 2.1 U/g, and protease 124000 PU/g in 300ml of solutions containing 30g of example 1 soybean protein, it was made to react to them at 50 degrees C for 5 hours, and the peptide was created. Consequently, compared with prolyl proteinase and the peptide created by the protease which does not contain carboxypeptidase activity, it was admitted as follows that there was little bitterness.

The inside of ten panelists To the usual protease Prolyl proteinase Peptide created more Carboxypeptidase It creates by the included protease. Manpower which sensed the peptide bitterness carried out Ten persons Manpower which did not sense three-person bitterness Zero person Seven persons [0014] the enzyme preparations which contain prolyl proteinase 4.8 mU/g originating in Aspergillus oryzae FS 1-32 (Fermentation Research Institute mycoparasite No. 12193), and carboxypeptidase 2.1 U/g to 300ml of solutions containing peptide 30g which the commercial protease (made in formation [PUROCHIN AY: Yamato]) was made to act on example 2 soybean protein, and was created -- 0.116g -- adding -- 50 degrees C -- 5 hours -- warming -- the after organoleptic test was conducted. Consequently, as for what performed enzyme processing compared with what does not perform processing by this enzyme, reduction of bitterness was accepted as follows.

Inside of ten panelists Non-processed peptide Enzyme processing peptide Manpower which sensed bitterness Ten persons Two persons Manpower which did not sense bitterness Zero person Eight persons

[0015]

[Effect of the Invention] By using this invention, a peptide with little bitterness can be created easily. Moreover, reduction of bitterness is possible by making the enzyme preparations which contain prolyl proteinase and carboxypeptidase also to the peptide which bitterness generated act. The protein of food can be strengthened by adding a peptide with little such bitterness for various kinds of food, without spoiling the original taste which the food has.

[Translation done.]